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Prevalence of Multidrug Resistant Bacterial Isolates from Meat Processing Equipment and Abattoir Environment in Ado Ekiti

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Abstract: Bacteriological investigation was carried out on the meat processing equipments and environment where animals were slaughtered at the central abattoir in Ado Ekiti in this study. Samples were collected from equipments such as knives, buckets, tables etc used in meat processing from the central abattoir in Ado Ekiti and also from the environment of the abattoir using swab sticks. The bacteria isolated were subjected to various biochemical tests and were also subjected to antimicrobial susceptibility tests. The microorganisms isolated include: *Aeromonas beastiarum, Proteus mirabilis, Corynebacterium accolens, Citrobacter youngae, Pediococcus pentosaceus Proteus vulgaris* etc. It was observed that more bacteria were isolated from the table on which the carcasses were sectioned than from other sources. The isolates showed resistance to multiple antibiotics. This study concludes that unhygienic environment and state of meat processing equipments could be a source of infection to consumers.

Key words: Abattoir · Antimicrobial · Bacteriological · Carcasses

INTRODUCTION

Meat is a nutritious, protein-rich food which provides the nutrients needed to support the growth of many types of microorganisms [1, 2]. This attribute makes meat highly perishable, having a short shelf life unless preservation methods are used [2].

The microbiological quality of meat depends on the physiological status of the animal at slaughter, the spread of contamination during slaughter and processing, the temperature and other conditions of storage and distribution [3]. For instance, the unwashed hands of meat handlers who are infected themselves can be a source of contaminant to the meat. Meat can be contaminated at any stage in the production process such as during dressing, slicing or sectioning of the carcass, moreover they could exposed to cross contamination during refrigeration. So, any of these can be a risk for food borne disease [4].

Contaminated raw meat is one of the main sources of zoonotic food borne illness [5, 6] and food borne pathogens are the leading cause of illness and death in developed and developing countries with attending high cost medically and socially [7,8].

Antibiotic resistance levels are also elevated among food-borne pathogens such as in *Salmonella* and *Shigella* [9,10]. Though the link between drug resistance in bacteria contaminating food items and increased clinical cases of resistant infections not fully defined; so the presence of bacteria in food items and their environment might play a role in the spread of antimicrobial resistance amongst food-borne pathogens and other microorganisms [6,11].

Various efforts which are aimed at reducing contamination and hence the spread of disease in meat industries include use of "sanitary controls" on the farms, developments of hazard analysis and critical control points (HACCP) in meat handling industries [7, 12].

This study aims at isolating, characterizing and determining the antibiotic susceptibility of microorganisms isolated from equipments used in abattoirs and the environment of abattoirs.

MATERIALS AND METHODS

Isolation and Identification of Isolates: Thirty samples were collected from equipments such as knives, buckets, tables etc used in meat processing from the central

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\abattoir in Ado Ekiti and also from the environment of the abattoir (especially the drainage) using Hi-culture Transport swab. The swab sticks were labelled and transferred to the laboratory aseptically. The swab sticks were inoculated into peptone broth and incubated for 24 hours at 37 °C, after which subculturing was done on different media such as blood agar, manitol salt MacConkey and nutrient agar. The isolates obtained were purified by further subculturing and were observed for presumptive identification based on their morphological characteristics and various biochemical tests that included catalase, oxidase, hydrogen sulphide production, motility, indole, methyl red, urea, Voges-Proskauer and citrate utilization tests [13]

Antibiotic Susceptibility Test: The antimicrobial susceptibility test was performed according to Bauer and Kirby [14] using Kirby-Bauer disk diffusion test. Each isolate was inoculated into nutrient broth separately and incubated for 24 hours at 37°C. The broth were streaked using sterile cotton swabs on Mueller-Hinton Agar plates. Two groups of antimicrobial agents were used; group for the Gram positive and another for Gram negative bacteria. The Gram positive antibiotic discs contained the following antimicrobial agents: cotrimoxazole (COT), cloxacillin (COX), erythromycin (ERY), Gentamicin (GEN), augmentin (AUG), streptomycin (S), tetracycline (TET) and chloramphenicol (CH) while the Gram negative antibiotic discs contained the following antimicrobial agents: augmentin (AUG), ofloxacin (OFX), gentamicin (GEN), nalidixic acid (NA), nitrofurantoin (NIT), cotrimoxazole (COT), amoxicillin (AMX) and tetracycline (TET). Zones of inhibition were evaluated following the recommendations by CLSI [15].

RESULTS

Thirty-two isolates were obtained from the samples collected from the equipment used in meat processing and the environment of the abattoir. Six out of the isolates obtained were Gram positive while the remaining isolates were Gram negative. Figure 1 shows the frequency of the microorganisms isolated.

Figure 2 shows the frequency of occurrence of microorganisms based on the sources of contamination of meat in the abattoir. It was observed that more microorganisms were isolated from the table used in sectioning the carcasses of the animals than from other sources.

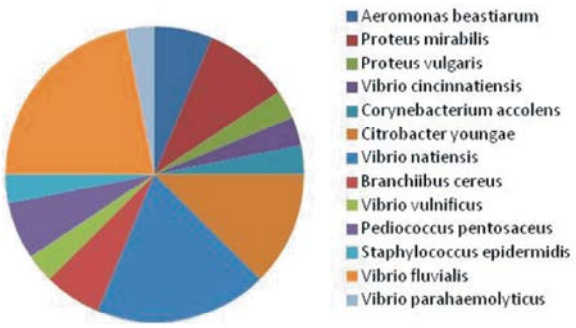

Fig. 1: Frequency of occurenc of mirroorgainisms (%)

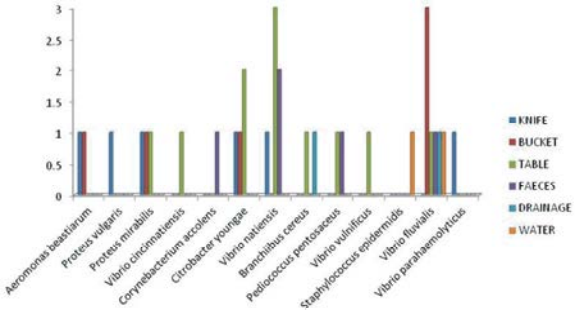

Fig. 2: Frequency of occurrence of microorgainisms based on the sources of contamination of meat in the abattoir

Tables 1 and 2 show the antimicrobial susceptibility of the Gram positive and Gram negative bacteria to Gram positive and Gram negative antibiotic discs respectively.

It was observed that cloxacillin and augmentin were the most resisted antibiotics among the Gram positive isolates since they were all resistant to these two antibiotics whereas in the Gram negative isolates,augmentin and cotrimoxazole were the most resisted antibiotics because all the Gram negative isolates were resistant to them. The Gram positive isolates showed the highest susceptibility to gentamicin with a percentage of 83.33 while the least susceptibility was to cotrimoxazole and tetracycline with 33.33% susceptibility respectively. Among the Gram negative isolates, nalidixic acid had the highest percentage of susceptibility with 80.77%, this was followed by ofloxacin with 76.92%, cotrimoxazole had the least percentage of susceptibility with 11.54%. All the isolates were resistant to multiple antibiotics showing different multidrug resistance (MDR) patterns. Some of the MDR patterns observed include: COT/COX/ERY/ AUG/S/TET/CH, COX/ERY/AUG, AUG/OFX/NIT/COT/ AMX/TET, AUG/NIT/COT/AMX/TET among others. Tables 3 and 4 shows the MDR patterns of the isolates based on the Gram reaction of the organisms.

Table 1: Antimicrobial susceptibility patterns of Gram positive bacteria.

	Antibiotics							
Isolates	Cotrimoxazole	Cloxacillin	Erythromycin	Gentamicin	Augmentin	Streptomycin	Tetracycline	Chloramphenicol
Branchiibus cereus (I)	R	R	R	S	R	R	R	R
Branchiibus cereus (II)	R	R	S	S	R	S	R	S
Corynebacterium accolens	S	R	R	S	R	I	R	R
Staphylococcus epidermidis	I	R	R	R	R	R	S	S
Pediococcus pentosaceus (I)	I	R	R	S	R	S	S	S
Pediococcus pentosaceus(II)	S	R	R	S	R	S	I	R
Susceptibility(%)	33.33	0	16.67	83.33	0	50	33.33	50

Keys: R- Resistanant (0), I- Intermediate (1), S- Susceptible (2)

Table 2: Antimicrobial susceptibility patterns of Gram negative bacteria.

	Antibiotics							
Isolates	Augmentin	Ofloxacin	Gentamicin	Nalidixic acid	Nitrofurantoin	Cotrimoxazole	Amoxicillin	Tetracycline
Aeromonas beastiarum (I)	R	R	S	S	R	R	R	R
Aeromonas beastiarum (II)	R	I	R	S	R	R	R	R
Proteus mirabilis (I)	R	S	S	S	I	R	R	I
Proteus mirabilis (II)	R	S	R	R	R	R	R	R
Proteus mirabilis (III)	R	S	R	S	R	R	R	R
Proteus vulgaris	R	S	R	S	S	R	R	R
Vibrio cincinnatiensis	R	S	R	S	S	S	R	S
Citrobacter youngae (I)	R	S	S	S	R	R	R	R
Citrobacter youngae (II)	R	R	R	S	R	R	R	R
Citrobacter youngae (III)	R	S	S	S	R	I	R	R
Citrobacter youngae (IV)	R	S	I	S	R	R	R	R
Vibrio natiensis (I)	R	I	R	R	R	R	R	R
Vibrio natiensis (II)	R	S	S	S	R	R	R	R
Vibrio natiensis (III)	R	S	S	S	R	R	R	R
Vibrio natiensis (IV)	R	S	S	S	S	S	R	I
Vibrio natiensis (V)	R	S	I	S	R	S	R	I
Vibrio natiensis (VI)	R	I	R	R	R	R	R	R
Vibrio vulnificus	R	S	R	S	R	R	R	R
Vibrio fluvialis (I)	R	S	S	S	S	R	R	S
Vibrio fluvialis (II)	R	S	R	S	S	R	R	R
Vibrio fluvialis (III)	R	S	R	R	S	R	R	I
Vibrio fluvialis (IV)	R	S	R	S	R	R	R	R
Vibrio fluvialis (V)	R	S	R	S	S	R	R	S
Vibrio fluvialis (VI)	R	I	R	I	I	R	R	R
Vibrio fluvialis (VII)	R	S	S	S	I	R	R	S
Vibrio parahaemolyticus	R	S	R	S	R	R	R	I
Susceptibility(%)	0	76.92	34.62	80.77	26.92	11.54	0	15.38

Keys: R- Resistanant (0), I- Intermediate (1), S- Susceptible (2)

Table 3: Multidrug Resistance (MDR) pattern of Gram positive bacteria

MDR pattern	No of organism
COT/COX/ERY/AUG/S/TET/CH	1
COT/COX/AUG/TET	1
COX/ERY/AUG/TET/CH	1
COX/ERY/GEN/AUG/S	1
COX/ERY/AUG	1
COX/ERY/AUG/CH	1

Table 4: Multidrug Resistance (MDR) pattern of Gram negative bacteria

MDR pattern	No of organism
AUG/OFX/ NIT/COT/AMX/TET	1
AUG/GEN/NIT/COT/AMX/TET	4
AUG/COT/AMX	1
AUG/GEN/NA/NIT/COT/AMX/TET	3
AUG/GEN/COT/AMX/TET	4
AUG/GEN	1
AUGNIT/COT/AMX/TET	4
AUG/OFX/GEN/NIT/COT/AMX/TET	1
AUG/NIT/AMX/TET	1
AUG/AMX	1
AUG/NIT/AMX	1
AUG/COT/AMX	2
AUG/GEN/NA/COT/AMX/TET	1
AUG/GEN/NIT/COT/AMX	1

DISCUSSION

Since most reports from different parts of the world has been concerned with the presence of bacteria in meat [16, 17]. This study evaluated the prevalence of multidrug resistant bacterial isolates from of equipment used in raw meat processing and the environment where raw meat is being processed.

The bacteria isolated in this study include *Aeromonas beastiarum, Branchiibius cereus, Proteus mirabilis, Corynebacterium accolens, Vibrio natiensis, Citrobacter youngae, Vibrio vulnificus, Pediococcus pentosaceus, Vibrio fluvialis, Proteus vulgaris, Vibrio cincinnatiensis, Staphylococcus epidermidis* and *Vibrio parahaemolyticus.* Most of these organisms have been implicated as pathogenic organisms [18,19].

Subsequently the presence of bacterial pathogens in meat-processing equipment and associated surfaces may contribute to the contamination of meat [6] and food-borne pathogens which are able to disseminate from contaminated meat to such surfaces [20] can spread infections in the community.

What is more, the isolated bacteria in this study showed varying degree of resistance to the different antibiotics used. The highest degree of resistance was observed among the isolated Gram negative bacteria. So the use of antibiotics rather than being an effective mean to prevent and control bacterial infection, their indiscriminate use can have adverse consequences by promoting the selection and prevalence of drug resistant microbial populations [21, 22] and be of great concern in relation to public health

According to Iroha *et al*. [10] the problem of antibiotic resistance in microorganisms may be due to the natural resistance of definite species to certain antibiotics, the transfer of antibiotic resistance among species and the use of sub-therapeutic doses of antibiotics in animal feeds to improve animal productivity, which could also result in the selection of resistant strains [23]. This is believed to be largely responsible for the emergence of drug resistance bacteria [24]. Piddock [25] suggested three possible ways in which the use of antibiotics could pose a risk to human health and these include: (a) selection of antibiotic resistant pathogens in animal which contaminates food product during slaughter and/or food preparation, the food is then ingested causing infection which requires antibiotic therapy and therapy is then compromised due to resistant strains; (b) resistant non-pathogenic bacteria are selected in animals which are transferred to humans via consumption of contaminated food products and resistant genes are subsequently transferred to other bacteria in the gut; (c) antibiotics which may remain as residues in animal products such as meat and milk can also lead to the selection of resistant bacteria in the consumer of the food products.

It was established through the findings of this study that the equipments used in processing meat in abattoirs and the abattoir environment could contaminate meat with different microorganisms including pathogenic ones. Hence, it is recommended that proper hygiene and good manufacturing practices be maintained in the abattoirs at all times. The abusive use of antibiotics in animal production should be discouraged so as to reduce the incidence of multi-drug resistance among microorganisms. It is also important to educate those working in the abattoirs on the need to maintain good hygiene both personally and also in their environment so as to reduce microorganisms that can contaminate the equipments used in the abatooir.

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